

## LISTING OF THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

### **Listing of Claims:**

1. (Withdrawn-previously presented) A method of culturing human embryonic stem (ES) cells with reduced differentiation comprising:

growing the human ES cells in culture, the cells proliferating in an unconditioned culture medium on a flexible solid porous matrix without fibroblast feeder cells in an apparatus configured to apply periodic strain to the matrix and the human ES cells.

2. -3. (Canceled).

4. (Withdrawn) The method of Claim 1 wherein the cell differentiation is eliminated.

5. (Withdrawn) The method of Claim 1 wherein the cells are grown on Matrigel™ using BioFlex® untreated culture plates.

6. (Withdrawn) The method of Claim 1 wherein the cells are grown without the presence of cross-species biological material.

7. (Withdrawn) The method of Claim 1 wherein the flexible matrix is Matrigel™.

8. (Withdrawn) The method of Claim 1 wherein the strain is mechanically produced.

9. (Withdrawn) The method of Claim 1 wherein the flexible matrix is stretched using vacuum pressure.

10. (Withdrawn) The method of Claim 1 wherein the strain exerted on the flexible matrix is at least about 5%.

11. (Withdrawn) The method of Claim 1 wherein the flexible matrix undergoes at least about 6 stretches per minute.

12. (Withdrawn) The method of Claim 1 wherein the mechanical strain is from oscillatory stretching of the flexible matrix surface.

13. (Previously presented) A cell culture composition comprising:  
human embryonic stem (ES) cells in culture, the cells proliferating in an unconditioned culture medium on a flexible solid porous matrix without fibroblast feeder cells in an apparatus configured to apply periodic strain to the matrix and the human ES cells.

14.-15. (Canceled).

16. (Previously presented) The culture composition of Claim 13 wherein substantially all of the human ES cells in the culture are undifferentiated.

17. (Previously presented) The culture composition of Claim 13 wherein the matrix comprises Matrigel™ and the apparatus comprises a BioFlex® untreated culture plate.

18. (Previously amended) The culture composition of Claim 13 wherein the culture medium is free of cross-species biological material.

19. (Previously presented) The culture composition of Claim 13 wherein the matrix comprises Matrigel™.

20. (Previously presented) The culture composition of Claim 13 wherein the apparatus is configured to apply mechanical strain to the matrix and the human ES cells.

21. (Previously presented) The culture composition of Claim 13 wherein the apparatus is configured to apply vacuum pressure to the matrix and the human ES cells.
22. (Previously presented) The culture composition of Claim 20 wherein the mechanical strain comprises oscillatory stretching.
23. (Previously presented) The culture composition of Claim 13 wherein the apparatus is configured to exert at least about 5% strain on the matrix.
24. (Previously presented) The culture composition of Claim 13 wherein the apparatus is configured to stretch the matrix at least about 6 times per minute.
25. (Withdrawn-Previously presented) A method of culturing human embryonic stem (ES) cells with reduced differentiation comprising:
  - a) growing the human ES cells in culture, the cells proliferating in an unconditioned culture medium on a flexible solid porous matrix without fibroblast feeder cells in an apparatus configured to apply periodic strain to the matrix and the human ES cells; and
  - b) applying an effective amount of periodic strain on the human ES cells, such that the human ES cells proliferate and exhibit reduced differentiation relative to human ES cells not subjected to periodic strain.
26. (Previously presented) The culture composition of Claim 13, wherein the human ES cells are characterized by positive expression of Oct4 and SSEA-4 cell surface markers.
27. (Previously presented) The culture composition of Claim 26, wherein the human ES cells are immuno-positive for alkaline phosphatase.

28. (Withdrawn - previously presented) A method of culturing undifferentiated human stem cells with reduced differentiation comprising:
- a) growing the undifferentiated human stem cells in culture on a flexible solid porous matrix without conditioned media and in the absence of fibroblast feeder cells in an apparatus configured to apply periodic strain to the matrix and the human ES cells, wherein the stem cells are defined by the positive expression of Oct4 and SSEA-4 cell surface markers; and
  - b) applying an effective amount of periodic strain on the flexible matrix to stretch the matrix and the undifferentiated stem cells thereon, such that the undifferentiated cells proliferate and exhibit reduced differentiation relative to undifferentiated human stem cells not subjected to periodic strain.